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Effects of calcium supplements on the quality and acrylamide content of puffed shrimp chips

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ABSTRACT

The quality and acrylamide content of deep-fried and microwave-puffed shrimp chips fortified with 0.1%, 0.5%, or 1.0% calcium salts (calcium lactate, calcium carbonate, calcium citrate, or calcium acetate) were investigated. Microwave-puffed shrimp chips contained higher amounts of acrylamide (130.43 ppb) than did deep-fried shrimp chips. The greatest mitigation of acrylamide formation in overfried chips was obtained with 0.1% calcium lactate. All browning indexes of fortified shrimp chips, whether deep-fried or microwave-puffed, were reduced. L^* values of microwave-puffed shrimp chips were higher than those of deep-fried shrimp chips, whereas a^* and b^* values and browning indexes were lower. Color differences (ΔE) between deep-fried puffed shrimp chips fortified with calcium salts and a control sample were higher than 5, and the sensory scores of shrimp chips were significantly decreased by the addition of calcium lactate.

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1. Introduction

Puffed shrimp chips, commonly known as *krupuk*, are a common snack in most Asian countries, including Taiwan, Indonesia, and Thailand. Raw shrimp chips are traditionally manufactured by mixing shrimp, starch, and water to form a paste, which is steamed, cooled down, cut into chips, and sun-dried. Raw shrimp chips are quite hard and are smaller and darker than puffed shrimp chips. Raw shrimp chips have been

traditionally puffed by deep-frying; however, microwave-puffed products are currently available in the market for health-conscious consumers.

Calcium is an essential nutrient that is recognized for its ability to prevent osteoporosis. The presence of polyvalent calcium ions in foodstuffs reduces acrylamide formation during heating [1]. Therefore, fortifying shrimp chips with calcium salts might theoretically reduce the formation of acrylamide during deep-frying or microwave puffing. Investigating the quality of puffed shrimp chips and the acrylamide

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content produced during microwave puffing could help the food industry design more effective methods for reducing the formation of acrylamide and for improving the nutritional value of shrimp chips.

Acrylamide is a potentially carcinogenic, neurotoxic compound that is present in heat-processed starchy food products [2]. It can also be generated by oils and nitrogen-containing compounds, or when oils in food ingredients are heated to temperatures above 100°C. However, acrylamide has not been detected in unheated or boiled foods [3]. The fatty acid oxidation product acrolein ($\text{CH}_2=\text{CH}-\text{CHO}$) reacts with ammonia to form $\text{CH}_2=\text{CH}-\text{CHOH}(\text{NH}_2)$, which can convert into acrylamide either by reacting with asparagine or by oxidizing to form an *N*-glycoside, which converts into acrylamide via oxidation [4]. The actual mechanism of acrylamide formation from the reaction of a carbonyl-containing compound and asparagine in fried midoleic sunflower oil at a temperature of 205°C has been described [5]. Tareke et al [6] reported that food heated at elevated temperatures is the main source of acrylamide. Becalski et al [7] proposed that acrylamide may be generated by the rearrangement of nitrogen-containing components present in cooked food-stuffs. Acrylamide is formed from food components during heating as a result of the Maillard reaction between reducing sugars and amino acids [8,9]. Levels of acrylamide in fried and baked starchy foods range between 150 and 4000 µg/kg [3]; therefore, finding an effective way to reduce the formation of acrylamide in fried snack foods is an urgent issue in the food processing industry.

In general, heat-processed commercial protein-rich foods, such as fish, meat, and poultry, contain lower amounts of acrylamide than do carbohydrate-rich foods, such as French fries, potato chips, tortilla chips, cereals, and baked goods [3]. The formation of a Schiff base is considered to be the first reaction step. The reaction is initiated by the addition of nucleophilic asparagine to the partially positive carbonyl carbon of the dicarbonyl compound, followed by the loss of a proton from nitrogen and the addition of a proton to oxygen [9].

Several strategies for reducing the formation of acrylamide in heated foods have been proposed in recent years, including the following: adding divalent cations such as calcium salts [10,11]; replacing reducing sugars with nonreducing sugars such as sucrose [12–14]; diluting asparagine levels by adding glycine [15]; adding asparaginase to reduce free asparagine [16,17]; and substituting ammonium salts with baking powder [18].

Jung et al [19] demonstrated that acrylamide formation in corn chips (baked and fried) and French fries was reduced when an acidulant was added to lower pH values. Amrein et al [14] suggested that free asparagine levels are a limiting factor in acrylamide formation.

To better understand the interactions that occur when fortified shrimp chips are deep-fried, we supplemented the standard chips recipe with calcium lactate, calcium carbonate, calcium citrate, or calcium acetate in order to determine the roles of calcium salts and reducing sugars in the formation of acrylamide. The quality of traditional puffed shrimp chips versus shrimp chips fortified with calcium salts was evaluated in order to develop better strategies for reducing the acrylamide content of snack products.

2. Materials and methods

2.1. Raw materials and chemicals

Cassava starch was obtained from Ding Yuh Foods (Taichung, Taiwan). Frozen white shrimp, sucrose, sodium chloride, and soybean oil were purchased from the local Ren Ai Traditional Market (Keelung, Taiwan).

3,5-Dinitrosalicylic acid, D-glucose, sulfuric acid, potassium sodium tartrate, $^{13}\text{C}_3$ -labeled acrylamide, and sodium hydroxide were purchased from Sigma Aldrich (St. Louis, MO, USA). All calcium salts (including calcium lactate, calcium acetate, calcium carbonate, and calcium citrate) were purchased from Nihon Shiyaku Industries, Ltd. (Taipei, Taiwan). All of the chemical reagents used were of analytical grade. The standard chemical compound 99.9% acrylamide was purchased from J.T. Baker (Phillipsburg, NJ, USA). Oasis HLB (6 mL, 200 mg) and Oasis MCX (3 mL, 60 mg) solid-phase extraction cartridges were obtained from Waters (Milford, MA, USA).

2.2. Preparation of raw shrimp chips and physicochemical properties of shrimp chips

The formula for raw shrimp chips is 200 g of white shrimp, 5.0 g of sodium chloride, 5.0 g of sucrose, 100 mL of distilled water, and 200 g of cassava starch. The base ingredients were blended with a 12-speed blender (Oster 6642; Oster, Canton, OH, USA). The resulting paste was supplemented with calcium salts (calcium lactate, calcium citrate, calcium acetate, or calcium carbonate) on a paste weight basis of 0.1%, 0.5%, or 1.0%. The shrimp paste was rolled out to a thickness of 20 mm, steamed for 1.5 hours, cooled down, refrigerated at 7°C for 8 hours, sliced into 3-mm chips, and dried at 80°C for 4 hours. The raw shrimp chips were either deep-fried in 500 mL of soybean oil at 180°C for 20–75 seconds in an electrical fryer (KR-4K009; Korlea Corporation, Kaohsiung, Taiwan) or puffed in a microwave oven (NN-GD587; Panasonic, Shanghai, China) for 40–80 seconds at 900 W.

The effects of calcium salts on the pH of raw shrimp chips were measured using the slightly modified Sung method [20]. The approximate chemical composition of puffed shrimp chips was determined according to the method of the Association of Official Analytical Chemists [21]. The moisture content of raw shrimp chips was measured according to Association of Official Analytical Chemists method 984.25, using oven drying at 105°C for 24 hours. The lipid content of the sample was determined using an ether extraction method [21]. The ash content of puffed shrimp chips was measured according to procedure 46-12 of the American Association of Cereal Chemists [22]. Water activity in puffed shrimp chips was determined using a Novasina Thermoconstanter RTD 33 TH-1 avumeter (Novasina Co. Ltd., Pfaffikon, Switzerland). The puffing ratio was calculated by measuring shrimp chips before and after puffing, using a sucrose granule displacement method. The puffing ratio was calculated as:

$$(V_2/W_2) \times 100\% / (V_1/W_1)$$

where W_1 = weight of raw shrimp chips; V_1 = volume of raw shrimp chips; W_2 = weight of puffed shrimp chips; and V_2 = volume of puffed shrimp chips.

2.3. Determining the color and browning index of puffed shrimp chips

The color of puffed shrimp chips was examined on a spectrophotometer (TC-1800 MK II; Tokyo Denshoku Co., Tokyo, Japan) using the L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness) color scale. Both a white tile and a black cup were examined before the test to standardize the spectrophotometer. The color of puffed shrimp chips was recorded after taking three measurements for each sample, and triplicate determinations were recorded for each treatment. Color difference (ΔE) was calculated using the following equation:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

where $\Delta L^* = L^*_{\text{sample}} - L^*_{\text{control}}$; $\Delta a^* = a^*_{\text{sample}} - a^*_{\text{control}}$; and $\Delta b^* = b^*_{\text{sample}} - b^*_{\text{control}}$.

The browning index (BI) was calculated using the following equation:

$$BI = 100 [x - 0.31]/0.17$$

where $x = (a^*_{\text{sample}} + 1.75L^*_{\text{sample}})/(5.645L^*_{\text{sample}} + a^*_{\text{sample}} - 3.012b^*_{\text{sample}})$.

L^*_{sample} , a^*_{sample} , and b^*_{sample} values correspond to the Commission internationale de l'éclairage color parameters of puffed shrimp chips made with varying amounts of calcium salts (0.1%, 0.5%, and 1.0%).

2.4. Assay for reducing sugars and amino acid analysis

A sample of finely ground shrimp chips (0.1 g) was added to 10 mL of 1.5M sulfuric acid and heated in a boiling water bath for 20 minutes. Once cooled, 12 mL of 10% sodium hydroxide was added. The solution was filtered and topped up to 100 mL with deionized distilled water. A standard glucose solution was used in the assay for reducing sugars. Reducing sugars were quantitatively measured using a dinitrosalicylic acid–reducing sugar assay, based on a slightly modified method proposed by Ilyina et al [23].

For amino acid analysis, puffed shrimp chips (2.5 g) were blended with 15 mL of 7% trichloroacetic acid (TCA) solution and centrifuged at 4000 rpm (GR21; Hitachi High-Technologies Corporation, Tokyo, Japan) for 20 minutes at 4°C. The supernatant was filtered with filter paper number 2 (Toyo Roshi Kaisha, Ltd., Tokyo, Japan). Pellets were blended with 7% TCA solution at 4°C and centrifuged as described previously. These procedures were performed twice. The supernatant was isolated by liquid–liquid extraction using an equal amount of ether as solvent to remove TCA. This procedure was performed five times. The extracted ether solution was evaporated under vacuum (vacuum decompression concentrator RV 10 basic; IKA, Staufen, Germany). Extracts were dissolved in 20 mL of double-distilled water. One milliliter of extracted solution was diluted to the appropriate concentration with 0.02N HCl, filtered through a nylon filter (0.22 μm), and injected using a mass spectrometer (AB Sciex QTRAP 5500; Agilent Technologies, Santa Clara, CA, USA). The solution was analyzed using an L-8900 amino acid analyzer (Hitachi High-Technologies Corporation).

2.5. Method for measuring and identifying acrylamide in puffed shrimp chips

Puffed shrimp chips were ground with a pulverizer (D3V-10; Yu Chi Machinery Co., Ltd., Chang Hua, Taiwan), and a 1-g sample was placed into a 50-mL centrifuge tube. Fifteen milliliters of deionized distilled water was added, and the solution was mixed in a 50°C reciprocal shaker bath for 60 minutes. The mixture was centrifuged at 9000 rpm for 20 minutes at 5°C. The supernatant was filtered through a nylon filter (0.45 μm). The Oasis HLB/MCX cartridge was conditioned with 5 mL and 3 mL of methanol, followed by 5 mL and 3 mL of deionized distilled water, respectively. The filtrate (1.5 mL) was passed through the Oasis HLB/MCX cartridge to absorb acrylamide and was discarded. The cartridge was washed with 3.5 mL of deionized distilled water; the first 0.5 mL of filtrate was discarded, whereas the remaining 3.0 mL of eluent was collected in a glass tube. The eluent was concentrated under vacuum for high-performance liquid chromatography (HPLC) and mass spectrometry (MS) analyses.

Acrylamide levels of shrimp chips were determined using HPLC [8]. The HPLC system (D2000) consisted of an L-2130 pump, an L-2400 detector, an L-2300 column oven, and an L-2200 autosampler (Merck Hitachi, Kent, UK). Chromatographic separation was performed in a Capcell Pak C₁₈ AQ S5 column (5 μm , 4.6 mm \times 250 mm; Shiseido, Tokyo, Japan) using deionized distilled water at 25°C at a flow rate of 0.7 mL/min (mobile phase: 100% double-distilled water; injection volume: 20 μL). The 400- μL sample was spiked with 100 μL of $^{13}\text{C}_3$ -labeled acrylamide with 0.5 mL of double-distilled water. The acrylamide eluate (2 μL) of HPLC was injected into an MS detector [mobile phase: (a) 0.1% (vol/vol) formic acid/water; (b) 0.1% (vol/vol) formic acid/methanol; source temperature: 600°C; electrospray capillary voltage: 5.5 kV; collision energy: 30 V]. Data acquisition was performed using the selected ion monitoring mode. The ions monitored in the sample had m/z values of 72.04 and 75.04 for acrylamide and $^{13}\text{C}_3$ -labeled acrylamide, respectively. Full-scan analyses were performed within the mass range 50–200. The acrylamide calibration curve was built within the range of 5.208–500 ng/g.

2.6. Sensory evaluation of shrimp chips fortified with calcium lactate

Fourteen male and 33 female students and faculty members of the Department of Food Science, aged between 20 and 55 years, were recruited as panelists. Shrimp chips fortified with calcium lactate (0.1%, 0.5%, or 1.0%) were served to the untrained sensory panelists. Chip samples were coded in three digits; panelists were asked to evaluate their aroma, color, and texture, and to provide an overall score using a 7-point hedonic scale (1 = “extremely dislike” to 7 = “extremely like”). Each data point from the sensory analysis represents the average of the panelists' opinions.

2.7. Statistical analysis

We used a completely randomized block design with three replications per treatment. Data were analyzed with analysis of variance using SPSS version 1.2 (1998; SPSS Inc., Chicago, IL,

USA). The Duncan multiple range test was used to identify the difference between treatments at a 5% significance level ($p < 0.05$).

3. Results and discussion

3.1. pH of puffed shrimp chips

The pH values of deep-fried and microwave-puffed shrimp chips were 6.48 and 6.46, respectively. The addition of 0.5% calcium lactate, calcium citrate, or calcium acetate to the shrimp chip formulation significantly decreased pH in puffed shrimp chips produced by deep-frying and microwave puffing. However, the addition of calcium carbonate did not affect the pH of shrimp chips, probably because the amino group reacted with a carbonyl source in cassava starch during Maillard reaction, resulting in elimination of water. Hydrogen ions were released by Amadori rearrangement, which decreased the pH of shrimp chips. Clawson and Taylor [24] reported that the pH of two ground wheat samples, cooked in sealed glass ampoules for 70 minutes at 121°C, decreased from the initial values of 6.12 and 7.81 to 5.88 and 6.32, respectively. Hamlet and Sadd [25] found that pH dropped from 5.91 to 4.73 in a pressure-cooking apparatus where basic flour, water, and salt dough chips were cooked at 180°C for 20 minutes.

The divalent calcium ion was able to induce a significant decrease in the pH of cookie dough, leading to a reduction in acrylamide formation [26]. The calcium ion-induced lowering of pH may have been caused by the competitive displacement of protons from ionizable oxygen, nitrogen, or sulfur atoms, which share electrons with hydrogen atoms. As a consequence, mass action competition occurs between protons and calcium ions when the pH is high enough; when the pH is not as high, most protons are removed prior to the addition of calcium ions [27].

3.2. Physicochemical properties of traditional shrimp chips and chips fortified with calcium supplements

The puffing ratio for deep-frying increased to a maximum of 745% with 0.1% calcium carbonate fortification. The puffing ratio for microwave puffing increased to a maximum of 1063% with 0.1% calcium acetate fortification. Calcium acetate induced a higher puffing ratio for microwave-puffed shrimp chips compared with all other forms of calcium. In general, the puffing ratio for microwave-puffed shrimp chips fortified with 0.1% calcium salts was higher than that for control.

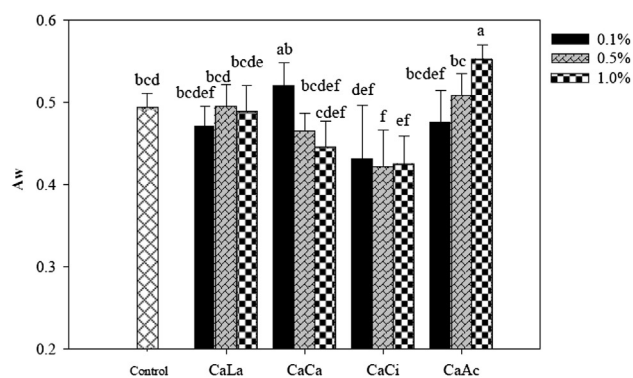
There was no correlation between the puffing ratio and the moisture content of raw shrimp chips ($R^2 = 0.0009$). There was, however, some correlation between the puffing ratio and the fat content of deep-fried puffed shrimp chips ($R^2 = 0.30$). The addition of calcium salts decreased the puffing ratio for shrimp chips, perhaps because the low pH and high amount of calcium resulted in the formation of shrimp paste, which mitigated chip puffing during frying.

The puffing ratio for shrimp chips fortified with 0.1% calcium salts was higher than that for the control sample, indicating that a low binding strength developed once a strong paste network had formed after calcium salts were added.

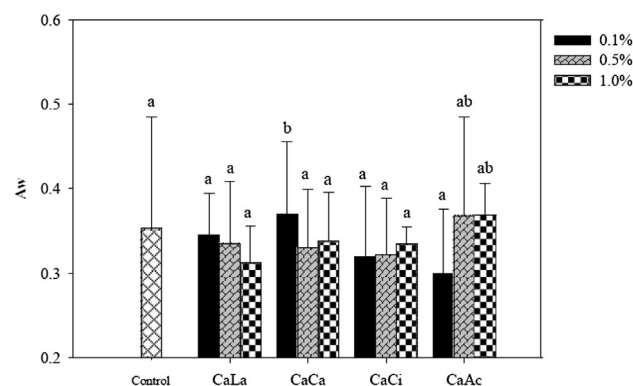
Additional calcium salts might have toughened the paste by interacting with proteins, thus preventing its expansion.

In this study, water activity in microwave-puffed shrimp chips was not decreased by calcium salt fortification, as it was designed to eliminate the possibility of acrylamide formation arising from the reaction between reducing sugars and nitrogen-containing compounds. Water activity in deep-fried and microwave-puffed shrimp chips in the control sample was 0.49 and 0.35, respectively (Fig. 1). Reduction of water activity was only detected in deep-fried puffed shrimp chips fortified with calcium citrate. From a food safety prospective, the levels of water activity in all control and fortified chips were low enough for either deep-frying or microwave puffing. Compared with the control sample, the addition of calcium salts at a concentration higher than 0.1% significantly increased the ash content of shrimp chips ($p < 0.05$). Our results suggest that the interaction between calcium cations and reducing sugars in shrimp chips was the main factor affecting pH in shrimp chips.

Moisture loss in raw shrimp chips during oven drying at 80°C for 240 minutes shows a typical dehydration profile.



(A) Deep-fried puffed shrimp chips



(B) Microwave-puffed shrimp chips

Fig. 1 – (A) Effects of different kinds and amounts of calcium salts on water activity (A_w) in deep-fried shrimp chips. (B) Effects of different kinds and amounts of calcium salts on A_w in microwave-puffed shrimp chips. ^{a–f} Significant difference between different kinds and amounts of calcium salts ($n = 3$; $p < 0.05$). CaAc = calcium acetate; CaCa = calcium carbonate; CaCi = calcium citrate; CaLa = calcium lactate.

From the point of view of heat and water transfer, the drying curves of the control and calcium-fortified raw shrimp chips were quite similar.

Obvious changes in puffing ratio and color were observed once the raw shrimp chips were deep-fried. Lower a^* values, lower BI, and higher L^* values were found in chips fortified with calcium salts compared with control shrimp chips (Table 1). However, the color difference was less apparent in microwave-puffed products (Table 1).

Color differences (ΔE) between deep-fried calcium citrate-fortified shrimp chips and control shrimp chips were readily apparent and higher than 9 (Table 1). The difference in Commission internationale de l'éclairage $L^*a^*b^*$ values was easily noticed when calcium salts were added to the shrimp chip formulation. The color of deep-fried shrimp chips fortified with calcium salts was more greenish for b^* values. The darkest color among the chip samples was seen in the group treated with 1.0% calcium acetate. The BI of shrimp chips treated with 1.0% calcium acetate was more intense than that of shrimp chips treated with 1.0% calcium carbonate (Table 1). The degree of browning was not closely related to the formation of acrylamide.

All calcium salt-fortified groups produced puffed shrimp chips with lighter surfaces and interior browning. Our results were in agreement with those reported by Jung et al [19] (i.e., that the degree of browning in baked corn chips treated with

citric acid was not directly related to the concentration of acrylamide in corn chips).

3.3. Reducing sugars in puffed shrimp chips and chips fortified with calcium salts

Adding calcium salts increased the amount of reducing sugars in deep-fried and microwave-puffed shrimp chips. Gokmen and Senyuva [1] reported that when the concentration of calcium cations was increased, the amounts of reducing sugars and glucose in a glucose-asparagine model system decreased; however, a significant increase in the amount of reducing sugars in shrimp chips fortified with 0.1% and 0.5% calcium citrate was observed in this study, indicating this is not always the case in this model system. The presence of calcium in the reaction mixture influenced the type and characteristics of reaction products during heating, and calcium also influenced the rate of decomposition of reaction precursors. Gokmen and Senyuva [1] demonstrated that the presence of calcium cations reduced acrylamide concentration by increasing furfural and hydroxymethylfurfural concentrations. They postulated that the key intermediate for acrylamide formation—the Schiff base—was inhibited and shifted to another reaction pathway, with dehydration of glucose leading to hydroxymethylfurfural and furfural formation [1]. When the concentration of calcium increased, the

Table 1 – Commission internationale de l'éclairage $L^*a^*b^*$ values for deep-fried and microwave-puffed shrimp chips formulated with different kinds and amounts of calcium salts.

		L^*	a^*	b^*	ΔE	Browning index
Deep-fried puffed shrimp chips						
Calcium lactate	Control	81.71 ± 3.80 ^a	5.77 ± 1.19 ^a	71.32 ± 0.98 ^a	0.00	164.62
	0.1%	89.13 ± 0.37 ^{bcd}	0.15 ± 0.92 ^{bc}	66.34 ± 1.11 ^{ab}	10.56	120.27
	0.5%	90.05 ± 0.63 ^{cd}	−1.30 ± 1.52 ^{bc}	65.24 ± 2.73 ^b	12.52	113.67
	1.0%	87.61 ± 3.00 ^{bc}	−0.40 ± 2.64 ^{bc}	65.08 ± 10.57 ^b	10.57	119.36
Calcium carbonate	0.1%	87.22 ± 0.51 ^{bc}	1.85 ± 0.21 ^{bc}	68.46 ± 0.55 ^{ab}	7.35	113.17
	0.5%	88.79 ± 0.24 ^{bcd}	0.97 ± 0.28 ^{bc}	68.20 ± 0.30 ^{ab}	9.10	127.57
	1.0%	90.12 ± 0.24 ^{cd}	−1.80 ± 0.61 ^c	64.28 ± 0.92 ^b	13.34	110.33
	1.0%	88.93 ± 0.25 ^{bcd}	0.66 ± 0.35 ^{bc}	67.22 ± 0.58 ^{ab}	9.75	123.86
Calcium citrate	0.1%	88.92 ± 2.18 ^{bcd}	1.19 ± 4.66 ^{bc}	67.36 ± 6.91 ^{ab}	9.41	124.85
	0.5%	92.43 ± 0.19 ^d	−6.30 ± 0.33 ^d	56.19 ± 0.24 ^c	22.10	81.81
	1.0%	89.95 ± 0.87 ^{cd}	−0.98 ± 2.24 ^{bc}	65.15 ± 3.75 ^b	12.32	113.93
	1.0%	85.50 ± 1.75 ^b	2.20 ± 0.94 ^{abc}	68.82 ± 0.98 ^{ab}	5.78	139.39
Calcium acetate	1.0%	80.05 ± 1.74 ^a	2.58 ± 0.30 ^{ab}	67.75 ± 0.59 ^{ab}	5.07	152.79
Microwave-puffed shrimp chips						
Calcium lactate	Control	94.29 ± 0.15 ^a	−8.90 ± 0.26 ^a	44.30 ± 0.63 ^a	0.00	51.18
	0.1%	95.11 ± 0.02 ^{ab}	−9.60 ± 0.07 ^{bcde}	37.47 ± 0.11 ^a	6.925	40.25
	0.5%	94.46 ± 0.09 ^a	−9.10 ± 0.22 ^{abc}	43.30 ± 0.80 ^a	1.043	51.05
	1.0%	95.25 ± 0.02 ^{ab}	−9.90 ± 0.11 ^{de}	36.82 ± 0.30 ^a	7.62	38.86
Calcium carbonate	0.1%	95.12 ± 0.08 ^{ab}	−9.70 ± 0.09 ^{cde}	37.37 ± 0.62 ^a	7.03	39.99
	0.5%	94.41 ± 0.56 ^a	−8.90 ± 0.82 ^{ab}	43.13 ± 3.92 ^a	1.18	50.92
	1.0%	95.03 ± 0.17 ^a	−9.70 ± 0.14 ^{cde}	38.50 ± 1.14 ^a	5.90	41.94
	1.0%	94.76 ± 0.51 ^a	−9.20 ± 0.48 ^{abcd}	40.42 ± 4.69 ^a	3.93	45.71
Calcium citrate	0.1%	94.87 ± 0.06 ^{ab}	−9.40 ± 0.07 ^{abcde}	39.92 ± 0.24 ^a	4.46	44.59
	0.5%	95.08 ± 0.28 ^b	−9.90 ± 0.10 ^e	34.22 ± 0.96 ^a	10.17	34.84
	1.0%	94.88 ± 0.10 ^{ab}	−9.50 ± 0.11 ^{abcde}	39.90 ± 2.19 ^a	4.48	44.54
	1.0%	94.89 ± 0.22 ^{ab}	−9.70 ± 0.19 ^{cde}	39.81 ± 2.41 ^a	4.61	44.14
Calcium acetate	1.0%	95.31 ± 0.31 ^{ab}	−10.00 ± 0.19 ^e	36.08 ± 2.82 ^a	8.37	37.56

Data are presented as mean ± SD.

a^* = redness/greenness; b^* = yellowness/blueness; ΔE = total color difference; L^* = lightness.

^{a–e} Values with different letters within the same column are significantly different at $p < 0.05$ ($n = 3$).

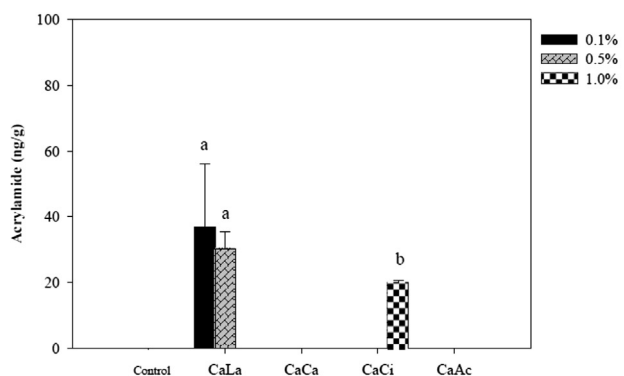
reaction proceeded mainly toward the dehydration of glucose, leading to hydroxymethylfurfural formation. Nevertheless, the presence of calcium ions increased the amount of reducing sugars and acrylamide formation in this study. Rydberg et al [28] confirmed that the presence of asparagine in 38mM fructose or 56mM glucose could increase acrylamide concentration in heated foods.

3.4. Addition of calcium salts and their effects on the formation of acrylamide

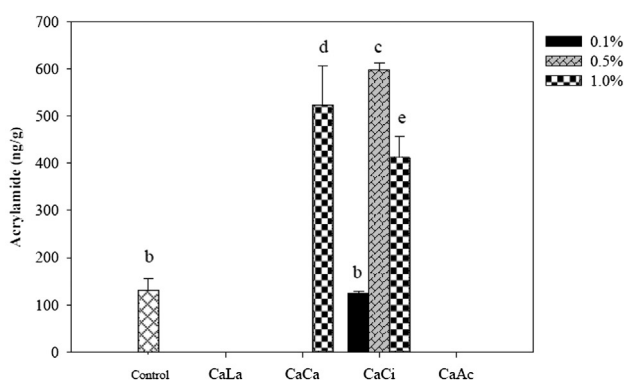
After 20 seconds of frying, acrylamide was detected in shrimp chips treated with 0.1% calcium lactate, 0.5% calcium lactate, and 1.0% calcium citrate, using HPLC and MS (Fig. 2). Puffed shrimp chips that had no calcium salts added contained a nondetectable amount of acrylamide following 20 seconds of deep-frying and 130.43 ng/g acrylamide after 50 seconds of microwave puffing (Fig. 2). After 20 seconds of deep-frying, a nondetectable amount of acrylamide in shrimp chips treated with calcium carbonate and calcium acetate showed that the

first step in acrylamide generation had not yet occurred. Conversely, shrimp chips treated with 0.1% and 0.5% calcium lactate contained 36.82 ng/g and 30.36 ng/g acrylamide, respectively, indicating that calcium lactate fortification induced acrylamide formation (Fig. 2A). The acrylamide content (20.14 ng/g) in deep-fried shrimp chips treated with 1.0% calcium citrate was also higher than that in the control group ($p < 0.05$) (Fig. 2A). The amount and type of calcium derivatives significantly influenced acrylamide formation in puffed shrimp chips. The addition of organic acid to cookie recipes might enhance the formation of acrylamide caused by the hydrolysis of sucrose to reducing sugars, as previously reported [12,13]. The degree of hydrolysis of sucrose to reducing sugars (fructose and glucose) determines the acrylamide concentration during baking. However, because the baking process is longer than deep-frying or microwave puffing, reducing sugars and free amino acid content—rather than the degree of sucrose hydrolysis—might be more important for deep-frying or microwave puffing. The reducing sugar content in shrimp chips fortified with calcium citrate was higher than that found in shrimp chips fortified with other calcium salts. The addition of calcium citrate may induce the formation of acrylamide (Fig. 2), especially in microwave-puffed shrimp chips.

Fig. 3 shows that the acrylamide concentration in microwave-puffed shrimp chips was higher than that in deep-fried shrimp chips, probably because microwave puffing required a longer heating time (50 seconds) than deep-frying (20 seconds) and generated more acrylamide (Fig. 2). The acrylamide concentration in cookies, as reported by Acar et al [8], was 128 ng/g, which was higher than that in deep-fried puffed shrimp chips; this may be attributed to either the difference in protein content or the longer heating time. The influence of heat processing time on the formation of acrylamide was reported in the study by Friedman [29], where the protein content of cake flour was diluted to less than 9%, whereas the protein content in our shrimp chip experiments was 12%. The cookie dough would have been baked at a higher temperature for a longer time, leading to an increased amount of acrylamide formed in the cookies. The significant decrease in acrylamide formation in the cookies caused by the addition of calcium carbonate and calcium citrate was not observed in this research with the addition of 1% calcium carbonate and calcium citrate to microwave-puffed shrimp chips (Fig. 2). In contrast, for the microwave-puffed shrimp chips control group, the increase in acrylamide content after fortification with 1.0% calcium carbonate and calcium citrate was dramatic (Fig. 2B). The results suggest that adding 1% calcium carbonate and more than 0.5% calcium citrate to microwave-puffed shrimp chips should be avoided. In deep-fried shrimp chips, a maximal acrylamide concentration of 53.13 ng/g was generated by overfrying (45 seconds of frying time) (Fig. 3). In microwave-puffed chips, however, the concentration of acrylamide dramatically exceeded 130 ng/g when the chips were overheated (Fig. 3); therefore, overheating during microwave puffing should be avoided. Although microwave-puffed shrimp chips contain less oil, they may form more acrylamide compared to deep-fried chips. Acar et al [8] showed that calcium salts significantly decreased acrylamide formation in cookies. Gokmen and Senyuva [1] reported

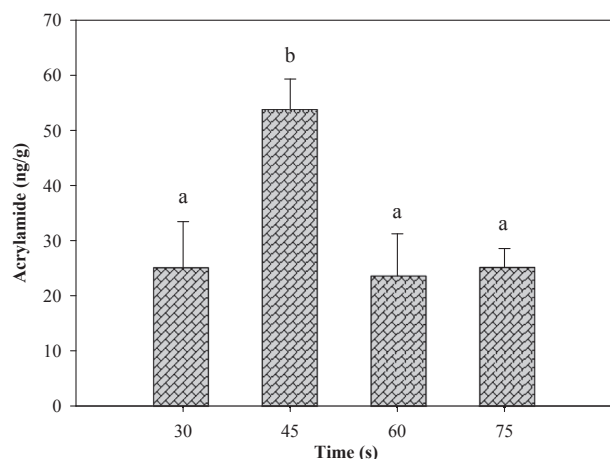


(A) Deep-fried puffed shrimp chips

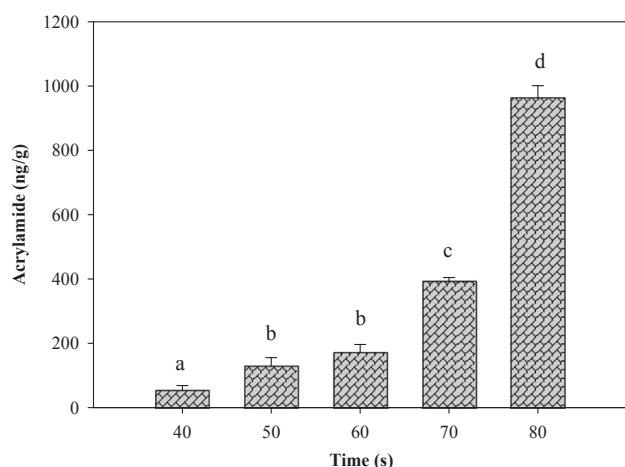


(B) Microwave-puffed shrimp chips

Fig. 2 – (A) Effects of different kinds and amounts of calcium salts on the amount of acrylamide in deep-fried shrimp chips (180°C, 20 seconds). (B) Effects of different kinds and amounts of calcium salts on the amount of acrylamide in microwave-puffed shrimp chips (900 W, 50 seconds).^{a–d} Significant difference between different kinds and amounts of calcium salts ($n = 3$; $p < 0.05$). CaAc = calcium acetate; CaCa = calcium carbonate; CaCi = calcium citrate; CaLa = calcium lactate.



(A) Deep-fried puffed shrimp chips



(B) Microwave-puffed shrimp chips

Fig. 3 – (A) Effects of time on the amount of acrylamide in deep-fried puffed shrimp chips. (B) Effects of time on the amount of acrylamide in microwave-puffed shrimp chips (900 W). ^{a–d} Significant difference between different amounts of calcium salts ($n = 3$; $p < 0.05$).

that adding calcium ions enhanced the stability of asparagine–matrix interactions at high temperatures, thus preventing asparagine from reacting with carbonyl precursors to form acrylamide during Maillard reaction. Sadd et al [30] found that fortification of bread dough with different calcium salts reduced the formation of acrylamide; however, this was not the same case with microwave-puffed shrimp chips.

In the overcooking tests conducted in this study, calcium salts were not effective in preventing the formation of acrylamide in deep-fried shrimp chips, except with 0.1% calcium lactate. The addition of 1% calcium acetate actually enhanced the formation of acrylamide (Fig. 4); therefore, the addition of calcium salts seems to be a less important factor in preventing acrylamide formation than are the choice of puffing method and the avoidance of overcooking. Acar et al [8] observed that a lower amount of calcium and a higher amount of lactate increased acrylamide formation. Raw shrimp chips fortified

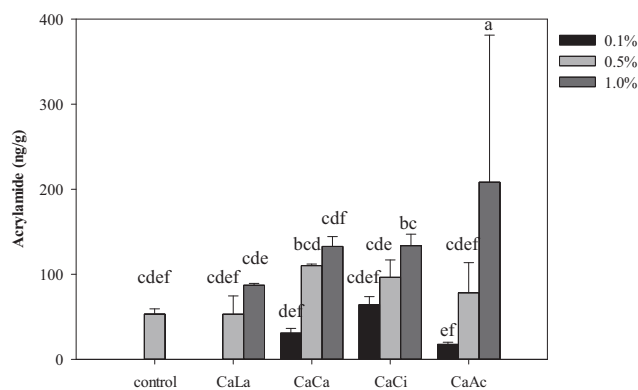


Fig. 4 – Effects of different kinds and amounts of calcium salts on the amount of acrylamide in overfried shrimp chips (180°C, 45 seconds). ^{a–f} Significant difference between different kinds and amounts of calcium salts ($n = 3$; $p < 0.05$). CaAc = calcium acetate; CaCa = calcium carbonate; CaCi = calcium citrate; CaLa = calcium lactate.

with 0.1% and 0.5% calcium salts had an increased tendency to form acrylamide during deep-frying ($p > 0.05$).

The acrylamide concentration in overfried shrimp chips fortified with 0%, 0.1%, 0.5%, and 1.0% calcium lactate was 53.0 ng/g, 0 ng/g, 53.0 ng/g, and 87.07 g/g, respectively, demonstrating that fortification with 0.1% calcium lactate can completely mitigate acrylamide formation ($p < 0.05$) (Fig. 4). The acrylamide concentration in overfried shrimp chips was not significantly different when they were fortified with 0.1% of any of the other calcium salts ($p > 0.05$). When the concentration of calcium increases, reaction proceeds mainly towards the dehydration of glucose, leading to hydroxymethylfurfural as one of the characteristic end products. This trend, however, was not observed in our results.

Although the use of 0.1% calcium carbonate, calcium citrate, and calcium acetate also decreased acrylamide formation in overfried shrimp chips, calcium lactate proved to be the most effective ($p < 0.05$).

In this research, deep-frying or microwave puffing shrimp chips for a longer time was the main contributing factor in acrylamide formation. Although fortification with 0.1% calcium lactate may mitigate some of the effects, the best option is to deep-fry—rather than microwave puff—the chips for an appropriate length of time.

3.5. Correlation between the amount of reducing sugars and acrylamide content in puffed shrimp chips

The correlation between the amount of reducing sugars and acrylamide content in puffed shrimp chips was tested using calcium lactate ($R^2 = 0.52$), calcium citrate ($R^2 = 0.99$), calcium acetate ($R^2 = 0$), and calcium carbonate ($R^2 = 0$) in different amounts. The results showed that the predicted relationship between the level of acrylamide formation and the reducing sugar concentration was dependent on the calcium salts used to fortify the chips. Puffed shrimp chips fortified with calcium citrate, for instance, had the highest correlation. The

predictability of acrylamide formation with specific calcium salts was also reported by Amrein et al [14]. The equation could serve as a practical tool for the snack food industry, making it easier for manufacturers to predict the formation of acrylamide based on the presence of reducing sugars in foods fortified with calcium citrate. Although there was a positive relationship between the formation of acrylamide and the reducing sugar concentration in calcium lactate–fortified chips ($R^2 = 0.52$), the correlation coefficient was lower than that for shrimp chips fortified with calcium citrate. The low correlation could be attributed to the complete absence of acrylamide in fried shrimp chips. As the ingredients and frying conditions in the snack industry are normally kept consistent, the reducing sugar concentration in chips fortified with calcium lactate should not be used to predict acrylamide formation.

The reducing sugar concentration in puffed shrimp chips fortified with calcium carbonate and calcium acetate showed no correlation with acrylamide concentration ($R^2 = 0$), probably because calcium carbonate cannot be easily dissolved in water. The regression equation for deep-fried shrimp chips fortified with calcium citrate could possibly serve as a simple predictive method for determining the mitigation of acrylamide formation within the tested concentrations. Once the reducing sugar concentration is established, acrylamide could be reduced by selecting calcium salts and employing a minimal amount of reducing sugars or by increasing the concentration of calcium citrate.

The free asparagine levels in deep-fried puffed shrimp chips cooked for 20 seconds and 45 seconds are 106.94 μ M and 50.49 μ M, respectively. The lower level of free asparagine in fried shrimp chips cooked for 45 seconds may be attributed to their reaction with reducing sugars to form acrylamide. Although aspartic acid and glutamic acid are the stronger calcium binders among amino acids, they are still rather weak [31]. If aspartic acid and glutamic acid bind calcium very strongly, less calcium can react to cause dehydration of glucose, leading to hydroxymethylfurfural formation and generation of more acrylamide. The acrylamide level (undetectable) in shrimp chips fried for 20 seconds is lower than that in shrimp chips fried for 45 seconds (53.13 ppb). Other free amino acids, including alanine, arginine, glutamine, threonine, and valine, can generate small amounts of acrylamide [3], which are greater in shrimp chips fried for 20 seconds. Overfrying is a more critical factor in generating acrylamide than is the presence of free amino acids. Fortification of microwave-puffed shrimp chips had no significant

effects on reducing sugars regardless of the amount of calcium salt used.

3.6. Sensory evaluation of deep-fried puffed shrimp chips fortified with calcium lactate

Calcium lactate (0.1%) can significantly mitigate acrylamide formation during overfrying of puffed shrimp chips. Sensory evaluation was conducted on deep-fried calcium lactate–fortified puffed shrimp chips. There were significant differences in color, texture, aroma, and overall score between the control shrimp chips and those fortified with varying amounts of calcium lactate (Table 2). That said, the sensory panelists could not differentiate between the puffed shrimp chips fortified with 0.1% calcium lactate and the chips in the control group. The addition of calcium lactate (0.1%) to shrimp chips decreased the texture, aroma, odor, and overall score. The sensory panelists preferred crispy and red shrimp chips. Of the calcium salts evaluated in this study, 0.1% calcium lactate was the most effective in reducing acrylamide formation during overfrying; however, the puffed shrimp chips still contained 36.82 ppb of acrylamide. According to sensory evaluation results, 0.1% calcium lactate was the most acceptable concentration because 0.5% calcium lactate resulted in a less crispy texture and bitter taste. Our results correspond well with the report of Mestdagh et al [32], which stated that calcium lactate and calcium chloride at a concentration of 1% resulted in saltier and less sweet cookies compared with control cookies. Our results do not agree with the report of Acar et al [8], where addition of calcium carbonate reportedly produced a texture score higher than that of control cookies. The lowered quality of shrimp chips fortified with calcium salts indicates that this is not a good strategy for reducing acrylamide content in puffed shrimp chips.

4. Conclusion

Deep-frying generated less acrylamide in shrimp chips than did microwave-puffing; in fact, acrylamide levels in deep-fried puffed shrimp chips were undetectable. Calcium lactate at a concentration of 0.1% significantly reduced acrylamide formation in overfried chips. Calcium citrate can decrease water activity, thereby increasing the calcium content of puffed shrimp chips. Furthermore, the reducing sugar content in shrimp chips initially increased with the addition of calcium ions but gradually decreased when more calcium salts were

Table 2 – Effects of different amounts of calcium lactate on the sensory evaluation of deep-fried shrimp chips.

	Color	Texture	Flavor	Odor	Overall acceptability
Control	4.68 \pm 1.10 ^a	5.30 \pm 1.63 ^a	4.68 \pm 1.34 ^a	4.32 \pm 1.54 ^a	4.98 \pm 1.39 ^a
0.1%	4.57 \pm 1.13 ^a	3.23 \pm 1.20 ^b	4.06 \pm 1.29 ^b	3.72 \pm 1.19 ^b	4.00 \pm 1.19 ^b
0.5%	3.81 \pm 1.30 ^b	2.98 \pm 1.95 ^b	3.36 \pm 1.25 ^c	3.72 \pm 1.36 ^b	3.32 \pm 1.41 ^c
1.0%	3.87 \pm 1.11 ^b	3.55 \pm 1.73 ^b	2.53 \pm 5.48 ^d	3.26 \pm 1.30 ^b	3.13 \pm 1.50 ^c

Data are presented as mean \pm SD.

Sensory evaluation was based on a 7-point hedonic scale: 1 = “extremely dislike”; 4 = “neither like nor dislike”; 7 = “extremely like.”

^{a–d} Values with different letters within the same column are significantly different at $p < 0.05$ ($n = 48$).

added. Calcium fortification also produced lighter-colored shrimp chips. The formation of acrylamide showed a positive correlation with reducing sugar concentration in calcium lactate-fortified chips. The most effective was 0.1% calcium lactate, which reduced acrylamide formation by 100%, but 0.5% and 1% calcium lactate could worsen sensory acceptability for puffed shrimp chips. Therefore, supplementation with calcium salts should not be the top choice for the puffed shrimp chips processing industry. Instead, avoiding overcooking is a more effective way of preventing acrylamide formation.

Conflicts of interest

All authors have no conflicts of interest to declare.

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